

Amendments to the Specification:

Please replace paragraph [0024] with the following amended paragraph:

-- Figures 6A through 6E depict a phenotype comparison of non-activated monocytes into dendritic cells cultured in either ~~Teflon~~[®]-TEFLON[®] polytetrafluoroethylene (PTFE) bags or plastic tissue culture flasks in cell culture media supplemented with GM-CSF alone or GM-CSF plus IL-4 in the presence or absence of a dendritic cell maturation factor. Figure 6A depicts the percentage of cells that were CD1a positive. Figure 6B depicts the percentage of cells that were CD83 positive. Figure 6C depicts the relative level of expression (mfi) of CD80. Figure 6D depicts the relative level of expression (mfi) of CD86. Figure 6E depicts the relative level of expression (mfi) of HLA-DR. --

Please replace paragraph [0033] with the following amended paragraph:

-- In another embodiment of the invention, a metal chelator can be added to the culture media to further prevent or reduce the activation of the monocytic dendritic cells by chelating divalent cations, including for example, but not limitation, calcium ions. The use of low adherence or low-binding culture vessels can also reduce the avidity of attachment or binding of the dendritic cell precursors to prevent the cells from being activated. Particularly preferred low binding materials include, for example, but are not limited to, polypropylene, ~~Teflon~~[®] TEFLON[®], ~~PFTE~~ PTFE, and the like. The metal chelator can be used in combination with the blocking agents described above. --

Please replace paragraph [0067] with the following amended paragraph:

-- Briefly, CD14⁺CD1a⁻ monocytes were resuspended in either Iscove-modified Dulbecco's medium (IMDM, BioWhittaker) plus 2 mM L-glutamine (Gibco BRL) or X-VIVO-15[®] (BioWhittaker) culture medium plus 3% human serum albumin (HSA, Bayer). Cell suspensions were transferred into T-25 flasks (Greiner) and incubated for 30 minutes in a 6%

CO₂, 37°C incubator. After the incubation, human serum albumin (HSA) and granulocyte-macrophage colony-stimulating factor (GM-CSF, Immunex) were added to achieve a final concentration of 3% HSA and 500 units/ml GM-CSF. Both cultures were incubated for 4 days in a 6% CO₂, 37°C incubator. The surface expression of CD14 and CD1a were analyzed by use of labeled monoclonal antibodies specific for the molecules and detection using flow cytometry. Dotted histograms represented isotype control (background) staining (Figure 1).

Please replace paragraph [0069] with the following amended paragraph:

-- In another example, it was demonstrated that monocytes would differentiate *in vitro* into CD1a⁺ dendritic cells in the presence of GM-CSF alone when the cells were not allowed to form an adherence to a ~~Teflon~~[®] TEFLON[®] polytetrafluoroethylene (PTFE) culture bag. --

Please replace paragraph [0070] with the following amended paragraph:

-- Briefly, isolated monocytes from two leukapheresis donors[[,]] were independently resuspended in X-VIVO-15[®] (BioWhittaker) culture medium plus granulocyte-macrophage colony-stimulating factor (GM-CSF, Immunex) and human serum albumin (HSA, ~~Plasbumin~~[™] PLASBUMIN[™], Bayer) to achieve the final concentration 500 units/ml GM-CSF and 2% HAS, in the ~~Teflon~~[®] TEFLON[®] polytetrafluoroethylene (PTFE) bags. Cell suspensions in the bags were transferred to a 6% CO₂, 37°C incubator for 5 days. At the conclusion of the culture period, maturation agents (1:400 dilution of inactivated BCG (Organon-Teknika) and 500 U/ml IFN-γ (R and D Systems)) were added to the cultures. The maturation event was allowed to proceed for 4 hours. The surface expression of CD14 and CD1a on "live" cells was analyzed after forward-scatter (FS) and side-scatter (SS) gating with labeled monoclonal antibodies specific for the molecules using fluorescent activated cell flow analysis (Figures 2A and 2B). Isotype control antibodies were used as controls for background fluorescence and were IgG₁ for the antibody specific for CD1a and IgG_{2b} for the antibody specific for CD14. --

Please replace paragraph [0078] with the following amended paragraph:

-- In this example the phenotype of DCs cultured in either ~~Teflon~~ TEFLON[®] polytetrafluoroethylene (PTFE) bags or in flasks under various culture conditions were compared. The cells were grown in either GM-CSF alone or in GM-CSF supplemented with IL-4. A comparison was also made of the phenotype of cells that had or had not been exposed to maturation agents.

Please replace paragraph [0079] with the following amended paragraph:

-- Briefly, monocytes were resuspended at 1×10^6 cells/ml in X-VIVO-15[®] (BioWhittaker) culture medium and 2% HSA (Bayer) supplemented with 500 U/ml GM-CSF alone, or in GM-CSF in combination with 500 U/ml IL-4. Cell suspensions (duplicate bags for each culture condition[[s]]) were cultured in ~~Teflon~~ TEFLON[®] polytetrafluoroethylene (PTFE) bags (American Fluoroseal), or tissue culture flasks (GM-CSF/IL-4 combination only) in a 6% CO₂, 37°C incubator. After 5 days, maturation agents (1:400 dilution of inactivated BCG (Organon-Teknika) and 500 U/ml IFN- γ (R and D Systems)) were added to one of the duplicate ~~Teflon~~ TEFLON[®] PTFE bag cultures, as well as the flask culture. On day 6, all cultures were harvested. Their phenotypes were analyzed using staining with labeled monoclonal antibodies specific for CD80, CD83, CD86 and HLA-DR with detection by flow cytometry.

Please replace paragraph [0082] with the following amended paragraph:

-- Each of the populations of monocytes, activated and non-activated, were then incubated in X-VIVO-15[®] culture medium with 2% HSA in the presence of GM-CSF alone or in combination with IL-4 for 5 days. The resulting immature DCs were loaded with influenza A M1-A4 40mer peptide or keyhole limpet hemocyanin (KLH) for one hour prior to washing and maturing with BCG (1:400 dil) and IFN- γ (500 U/ml). After harvesting and washing the mature

DC, co-cultures with DCs and autologous PBMCs were set up at a 1:10 DC:PBMC ratio in AIM-V[®] culture medium plus 5% human AB sera (HuAB Sera supplemented with 20 ng/ml IL-2 from day 2 through day 8. After eight days of culture the T cell lines were harvested and analyzed for M1-A4 specific CD8 T cell expansion ($V\beta 17^+$ CD8⁺ T cells) by flow cytometry.

Please replace paragraph [0085] with the following amended paragraph:

-- Briefly, cryopreserved monocytes were previously isolated via a tangential flow filtration process from two different blood donors. This process comprised TFF of a sample of monocytes in a device having a filter with a pore size of 5.5 micron. The recirculation (input) rate was about 1400 ml/min, the filtration rate was about 17 ml/min, and the time was about 90 min. The enriched monocytic dendritic cell precursors were independently cultured at a concentration of 1×10^6 cells/ml in DC culture media containing X-VIVO-15[®] (BioWhittaker), 2% human serum albumin (Bayer), and 500 U/ml GM-CSF (Immunex). Cell suspensions in Teflon[®] TEFLON[®] polytetrafluoroethylene (PTFE) bags were cultured for 5 days in a 6% CO₂, 37°C humidified incubator. At the conclusion of the culture period, maturation agents (1:400 dilution of inactivated BCG (Organon-Teknika) and 500 U/ml IFN- γ (R and D Systems) were added to the cultures. The maturation event was allowed to proceed for 4 hours. Maturing DCs were harvested and characterized. The cells were reacted with labeled monoclonal antibodies specific for CD11c, CD1a, CD40, CD54, CD80, CD86, and CD83. Marker expression on "live" cells were analyzed by use of forward-scatter (FS) and side-scatter (SS) gating and with labeled monoclonal antibodies specific for the molecules and detection using flow cytometry. Greater than 80% of the cells recovered were of the monocyte lineage, that is CD11c expressing and were "live" DCs (propidium iodide⁻, not shown). Significantly, cells differentiated in the absence of IL-4 express the typical DC markers, *i.e.*, a decreased CD14 expression, and expression of CD1a, CD40, CD80, CD86, CD54, and CD83 (Figure 8A and 8B). Background fluorescence was measured using isotype control antibodies and were IgG₁, with the exception of CD14, where the isotype control was an IgG_{2b} antibody.

Please replace paragraph [0086] with the following amended paragraph:

-- In this example it was determined that monocytes that were exposed to plastic surfaces (*i.e.*, a tissue culture flask) became activated, unless tight interaction was blocked by the addition of a blocking agent, like human serum albumin-~~(HAS)~~ (HSA). --